

Guava Diseases in Hawaii and the Characterization of *Pestalotiopsis* spp. Affecting Guava

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Abstract

Guava (*Psidium guajava* L.), one of the most widely grown plants in the tropics, is very susceptible to disease which can decrease its marketability. Leaf and fruit spot diseases commonly occur on guava grown in Hawaii. A disease survey was conducted on more than 50 accessions grown at the USDA/ARS Tropical Plant Genetic Resource and Disease Research Unit in Hilo, Hawaii. The four main fungi isolated from leaves and fruit were *Pestalotiopsis*, *Colletotrichum*, *Mucor* and *Guignardia*. Disease symptoms of these fungi were visible on leaves without fruit present, and on the skin of young fruits (pinhead size) which progressed as fruits matured. The highest disease incidence by far (>85%) was for *Pestalotiopsis* spp. The main diagnostic symptoms were grey/light brown lesions surrounded by dark brown borders on leaves and brown, raised, corky, necrotic lesions on the exocarp of fruit. The *Pestalotiopsis* spp. were isolated, identified and characterized. Pathogenicity was demonstrated on wound-inoculated fruit and leaves by fulfilling Koch's postulates. Potential sources of host resistance were identified in the germplasm. The importance of *Pestalotiopsis* as a guava pathogen and its cross-infection potential are discussed.

INTRODUCTION

In Hawaii, common guava (*Psidium guajava* L.) is found throughout the island at various elevations and under various environmental conditions. Guava is also one of the most vigorous and widespread plants in the tropics and is associated with many fruit rot diseases. Guava is commonly processed into puree and juice, thus disease can decrease its marketability. According to Hawaii Agricultural Statistics Service (HASS), guava production utilized for processing totaled 1.7 million kg in 2007, with statewide farm values totaling \$597,000 (HASS, 2008).

It is common to see leaf and fruit spots on guava in Hawaii. Scabby fruit canker, caused by *Pestalotiopsis psidii*, was reported in India to drastically reduce yield in the field and affect post-harvest quality (Kaushik et al., 1972). Additional fruit rot diseases cause great losses in production (Lin et al., 2003). Identification and the proper management of the diseases are important for the successful cultivation of the crop. The objective of this work was to determine the most prevalent pathogens on guava in Hawaii and to determine if sources of host resistance to scabby canker exist in the available germplasm.

MATERIALS AND METHODS

Plants were examined in the fields of the Tropical Plant Genetic Resource and Disease Research Unit located at the Waiakea Agricultural Experiment Station, Hilo, Hawaii, and surrounding areas. Typical symptoms were noted and described. Disease severity data was collected and quantified. Disease severity scale was based on percent area of canopy showing typical symptoms of scabby canker (1 = 0 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = >76). Disease ratings were based on average visual observation for the entire tree. Potential sources of host resistance were identified and cultivars with severity ratings of 0 to 25% were considered resistant and 50% and higher were considered susceptible.

Plant material bearing lesions was collected and examined in the laboratory. Infected areas of leaves and fruit were excised, surface sterilized in 10% bleach (0.5% sodium hypochlorite) for one minute, placed on sterile Kimwipes to dry, and plated on water agar. After incubation for approximately 3 days at 24°C, advancing mycelium was transferred to potato dextrose agar (PDA) by hyphal tip transfer and monoconidial cultures were prepared and retained for all future experiments. The isolated fungi were identified based on morphological and cultural characteristics. After seven days on PDA, colony size, color, texture, color of conidial masses and zonation were noted by digital photography and described. Conidia were examined microscopically. Shapes and sizes were described. Plates were also incubated at various temperatures (ranging from 15 to 35°C) under continuous illumination to determine growth rates.

For molecular comparison of isolates, PCR reactions to amplify one or both of the ITS regions (1 and 2) were largely based on Caetano-Anolles et al. (2001) and White et al. (1990). PCR products were cloned (TA cloning kit, Invitrogen Co., San Diego, CA) and plasmid DNA for sequencing was prepared (Qiagen plasmid miniprep kit, Chatsworth, CA). DNA sequencing was performed at MWG Biotech Inc. (High Point, NC). Similarity searches of the GenBank database were performed with BLAST (Altschul et al., 1990).

Isolates were examined for their pathogenicity on leaves of *Psidium guajava* cv. 'Kona', 'Patillo' or 'Lucknow 49'. Fungal plugs or conidial suspensions of 5 to 7 day old cultures were used as inoculum. The fungi were re-isolated to fulfill Koch's postulates. Similar studies were conducted on the fruit of cultivars 'Lucknow' or 'Lucknow 49'.

A detached leaf bioassay was developed. Aluminum roasting pans (~ 30 x 50 cm) were used as inoculation chambers. The bottom of the pan was covered with four paper towels, with four inverted plastic petri dishes on top of the towels to keep the leaves from contact with the towels. Small, glass test tubes filled with sterile distilled water and covered with parafilm were placed in the pans. Leaf material was surface sterilized in 10% bleach and air dried in a laminar flow hood. The leaves were placed in the pan with the leaf twig in the glass test tube and the leaves supported by the petri dish. The agar plug was placed mycelia-side down between the main leaf vein and the leaf edge, and centered on one side of the leaf. Good contact with the leaf surface was ensured by pressing gently on the plug with forceps. The effects of wounding versus no wounding were compared. Approximately 150 ml of sterile distilled water was added to each pan, and then covered tightly with plastic film. For single leaves or leaf disks, Nunc bioassay plates or Petri dishes containing 250 ml or 25 ml water agar, respectively were used as inoculation chambers. We based the simple and reliable bioassay on the inoculation of detached, greenhouse-grown guava leaves, comparing lesion lengths on guava leaves resulting from inoculation with *Pestalotiopsis* spp. We compared age effect of the fungus on symptom development (5 days versus 14 days) and influence of cultivar on lesion size, using different varieties of guava and isolates of *Pestalotiopsis*. Detached young leaves and/or fruit of several crops (ohia, waiwi, rambutan, lychee, ginger, orchid, tea) were used to assess the pathogenic potential of the isolates of the *Pestalotiopsis* spp. For the leaf bioassay, lesion length <10 mm was considered resistant and >15 mm was considered susceptible.

RESULTS AND DISCUSSION

Typical symptoms of guava diseases observed in Hawaii can be seen in Figure 1. Disease symptoms were visible on the skin of young fruits (pinhead size) which progressed as fruits got larger. Symptoms of scabby canker caused by *Pestalotiopsis* spp. (A) included dark brown to black necrotic spots which developed into lesions with a corky appearance. This was the most prevalent disease observed (% incidence >85%). Minor diseases (% incidence <10%) included *Mucor* rot caused by *M. heimalis* (B) – water-soaked lesions covered with fuzzy mycelium; *Guignardia* fruit spots caused by *G. psidii* (C) - small, dark brown, necrotic spots; and Anthracnose (D), caused by *Colletotrichum gloeosporioides* – dark, depressed necrotic lesions with concentric circles and pink spore masses.

Scabby canker disease was the main focus of our study due to the prevalent nature of the problem. Visual observations in the field of disease severity on immature and mature fruit and leaves indicated that certain guava varieties exhibited potential sources of host resistance to scabby canker caused by *Pestalotiopsis* spp. (Table 1).

Our study identified and determined the cultural characteristics of the main species of *Pestalotiopsis* affecting guava using morphological and physiological comparisons (colony color and texture, conidial shape and length, color of conidial mass, growth rate, temperature response), established the pathogenicity and host range of the most consistently isolated species, and used molecular techniques (sequence and phylogenetic analysis of ITS region) to determine if a correlation existed between these methods and colony morphology-based classification. We collected more than 30 strains of *Pestalotiopsis* from guava and other crops which could serve as an inoculum source for scabby canker on guava (Keith et al., 2006). More than one species of *Pestalotiopsis* was capable of causing scabby canker on guava and also had the ability to cause leaf and/or fruit spots on alternative hosts such as ginger, rambutan, lychee and orchids. Typical cultural and morphological characteristics are shown in Figure 2. Optimum growth temperatures for all isolates were between 22 and 28°C. Phylogenetic analysis indicated a high level of identity among all of the *Pestalotiopsis* isolates (>90%). We compared different inoculation methods on disease severity of *Pestalotiopsis* on guava. All experiments confirmed that wounding enhanced timing and severity of symptoms. A more efficient screening method of germplasm for potential sources of resistance and a better understanding of the mechanisms contributing to disease resistance were needed, so we developed and evaluated a detached leaf assay. Results of the leaf bioassay indicated that wounding of leaves was most critical for symptom development. There was very little effect of fungal culture age on symptom development. Certain cultivars of guava showed varying susceptibility to the fungal pathogens (Table 2 and Fig. 3). There was a strong positive relationship between results of the leaf bioassay (Table 3) and field observations (Table 1) for isolates and cultivars tested. The new leaf bioassay method makes possible the rapid and non-destructive screening of large quantities of guava accessions for potential field resistance. The method offers prospects for quantifying the pathogenicity of different strains of *Pestalotiopsis* spp., assessing the relative susceptibility or resistance of different cultivars and germplasm, and identifying factors that affect guava host-*Pestalotiopsis* spp. interactions. Ultimately, this would promote the availability of resistant guava cultivars, which could benefit the growers by improving yields with higher quality plants as well as lower production costs. The leaf bioassay technique could also be utilized for other economically important crops.

CONCLUSIONS

Pestalotiopsis causing scabby canker on guava in Hawaii:

1. Ubiquitous in distribution
2. Causes considerable decay to fruits on the tree and post-harvest
3. Varied colony morphology
4. No correlation between morphology, sample type or location
5. Capable of growing at a wide range of temperatures (10 to 35°C)
6. Optimum growth temperature around 26°C

Detached leaf bioassay:

1. Simple and reliable
2. Wounding is necessary for infection
3. Guava cultivars showed varying susceptibility to fungal strains
4. Identified susceptible and resistant cultivars
5. Correlated leaf bioassay results to field observations

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Tables

Table 1. Results of survey indicating disease severity of scabby canker on guava fruit and leaves.

Cultivar	Immature fruit ^x	Mature fruit ^x	Leaves ^x	Resistance
Lucknow	3	3	3	
Fan Retief	-	-	2	
Puerto Rico #2	-	-	2	
Patillo	1	-	1	*
270	1	-	1	*
Kona	1	-	3	
507	3	4	2	
138-T	2	-	3	
Pink Acid	1	-	1	*
Beaumont	-	-	2	
Ka Hua Kula	2	3	3	
Waiakea	-	3	2	
Alahabad Safeda	1	2	2	*
Indian Red	3	3	2	
Uma	3	4	3	
Bon Dov	3	4	3	
Red Indian	1	2	1	*
157	3	4	4	
Poamoho Pink	2	-	3	
Hong Kong Pink	1	1	1	*
Gushiken Sweet	1	2	1	*
Jhao Sawaise	1	2	2	*
Pearl	2	-	2	

^x Disease severity scale is based on percent surface area showing typical symptoms of scabby canker.

1 = 0 to 25%; 2 = 26 to 50%; 3 = 51 to 75%; 4 = >76.

Disease rating is based on average visual observation for the entire tree

- = no fruit present, * = possible source of resistance

Table 2. Leaf bioassay parameters showing susceptibility of guava to scabby canker infection.

Cultivar	Isolate	Culture age	Wounding	Ratings*
Kona	P-17	5 days	Yes	4
Kona	P-17	5 days	No	1
Kona	P-17	14 days	Yes	4
Kona	P-17	14 days	No	1
Kona	P-20	5 days	Yes	4
Kona	P-20	5 days	No	1
Kona	P-20	14 days	Yes	3
Kona	P-20	14 days	No	1
270	P-17	5 days	Yes	3
270	P-17	5 days	No	1
270	P-17	14 days	Yes	3
270	P-17	14 days	No	1
270	P-20	5 days	Yes	2
270	P-20	5 days	No	1
270	P-20	14 days	Yes	2
270	P-20	14 days	No	1

Results are 7 days post inoculation.

* Lesion sizes: 1 = <2 mm; 2 = 2 to 3 mm; 3 = 3 to 6 mm; 4 = >6 mm

Table 3. Leaf bioassay results indicating potential sources of guava resistance to scabby canker disease.

Cultivar	Size of lesion (mm) ^y	Reaction ^z
Patillo	3.3 ± 1.1	R
Pink Acid	6.7 ± 2.2	R
Hong Kong Pink	9.0 ± 1.4	R
Kona	23.3 ± 6.2	S
270	5.4 ± 2.1	R
Gushiken Sweet	2.0 ± 0.0	R
Alahabad Safeda	3.8 ± 1.1	R
Red Indian	3.7 ± 1.0	R
Pearl	18.7 ± 1.2	S

Results are 8 days post inoculation with *Pestalotiopsis* isolate 20.

^y Each value is the mean diameter ± SEM

^z R = potential source of resistance (<10 mm); S = highly susceptible to infection (>15 mm)

Figures



Fig. 1. Most common disease symptoms caused by fungi on guava in Hawaii.
(A) *Pestalotiopsis*, (B) *Mucor*, (C) *Guignardia* and (D) *Colletotrichum*.

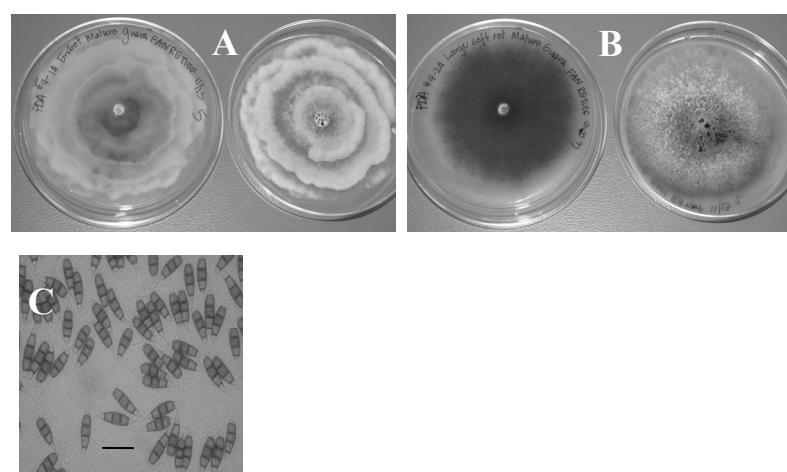


Fig. 2. Typical *Pestalotiopsis* colony characteristics on PDA after 7 to 10 days at 26°C.
(A) pale buff/pale saffron (reverse), pale buff (upper); (B) olivaceous buff (reverse), pale olivaceous buff (upper). (C) Commonly observed conidia from *Pestalotiopsis* isolated from guava. Bar = 25 μ m.

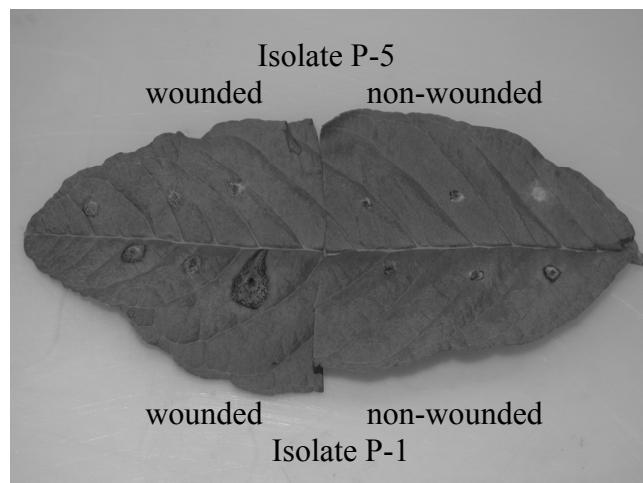


Fig. 3. Example of a detached leaf bioassay to screen guava for susceptibility to *Pestalotiopsis* spp. 'Kona', 7 days post-inoculation.

